PATENT
Attorney's Docket Number: 07681.0018-01
CUSTOMER NUMBER: 22,852

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

Prior Application: Art Unit: 1632 Examiner: D. Crouch

SIR: This is a request for filing a

■ Continuation □ Continuation-in-Part □ Divisional Application under 37 C.F.R. § 1.53(b) of pending prior application Serial No. 09/650,285 filed August 29, 2000 of Keith Campbell et al. for UNACTIVATED OOCYTES AS CYTOPLAST RECIPIENTS FOR NUCLEAR TRANSFER.

1. ■ Enclosed is a complete copy of the prior application including the oath or Declaration and drawings, if any, as originally filed. I hereby verify that the attached papers are a true copy of prior application Serial No. 08/803,165 as originally filed on February 19, 1997 .

2. □ Enclosed is a substitute specification under 37 C.F.R. § 1.125.

3. □ Cancel Claims ____.

4. ■ A Preliminary Amendment is enclosed.

5. ■ The filing fee is calculated on the basis of the claims existing in the prior application as amended at 3 and 4 above.

Basic Application Filing Fee					$740	$ 740.00
	Number of Claims		Basic	Extra Claims		
Total Claims	1	-	20		x $18	
Independent Claims	1	-	3		x $84	
□ Presentation of Multiple Dep. Claim(s)					+$270	
Subtotal						$ 740
Reduction by 1/2 if small entity						-
TOTAL APPLICATION FILING FEE						$ 740

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6. ■ A check in the amount of $740.00 to cover the filing fee is enclosed.

7. ■ The Commissioner is hereby authorized to charge any fees which may be required including fees due under 37 C.F.R. § 1.16 and any other fees due under 37 C.F.R. § 1.17, or credit any overpayment during the pendency of this application to Deposit Account No. 06-0916.

8. ■ Amend the specification by inserting before the first line, the sentence:

--This is a ■ continuation of application Serial No. 09/650,285 filed August 29, 2000, which is a continuation of application Serial No. 08/803,165, filed February 19, 1997, which claims the benefit of PCT/GB96/02098 , filed on August 30, 1996, and British application GB 9517779.6, filed on August 31, 1995, all of which are incorporated herein by reference.--

9. ■ New formal drawings are enclosed.

10. ■ The prior application is assigned of record to: Roslin Institute (Edinburgh) ; The Minister of Agriculture, Fisheries and Food; and Biotechnology & Biological Sciences Research Council.

11. ■ Priority of application Serial Nos. PCT/GB96/02098 , filed on August 30, 1996, and GB 9517779.6, filed on August 31, 1995 in Great Britain, is claimed under 35 U.S.C. § 119 or § 365. A certified copy

□ is enclosed or ■ is on file in the prior application.

12. □ A verified statement claiming small entity status
□ is enclosed or □ is on file in the prior application.

13. ■ An associate power of attorney in the prior application is to at least one of the following: FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P., Douglas B. Henderson, Reg. No. 20,291; Ford F. Farabow, Jr., Reg. No. 20,630; Arthur S. Garrett, Reg. No. 20,338; Donald R. Dunner, Reg. No. 19,073; Brian G. Brunsvold, Reg. No. 22,593; Tipton D. Jennings, IV, Reg. No. 20,645; Jerry D. Voight, Reg. No. 23,020; Laurence R. Hefter, Reg. No. 20,827; Kenneth E. Payne, Reg. No. 23,098; Herbert H. Mintz, Reg. No. 26,691; C. Larry O'Rourke, Reg. No. 26,014; Albert J. Santorelli, Reg. No. 22,610; Michael C. Elmer, Reg. No. 25,857; Richard H. Smith, Reg. No. 20,609; Stephen L. Peterson, Reg. No. 26,325; John M. Romary, Reg. No. 26,331; Bruce C. Zotter, Reg. No. 27,680; Dennis P. O'Reilley, Reg.

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14. ☐ The power appears in the original declaration of the prior application.

15. ■ Since the power does not appear in the original declaration, a copy of the power in the prior application is enclosed.

16. ■ Please address all correspondence to FINNEGAN, HENDERSON, FARABOW, GARRETT and DUNNER, L.L.P., 1300 I Street, N.W., Washington, D.C. 20005-3315. **Customer Number 22,852**.

17. ☐ Recognize as associate attorney _____

18. ■ Also enclosed is a Request Under 37 C.F.R. §1.607 for Interference with U.S. Patent 6,235,969 to Stice et al. with Exhibits A-E.

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PETITION FOR EXTENSION. If any extension of time is necessary for the filing of this application, including any extension in the parent application, Serial No. 09/650,285, filed August 29, 2000, for the purpose of maintaining copendency between the parent application and this application, and such extension has not otherwise been requested, such an extension is hereby requested, and the Commissioner is authorized to charge necessary fees for such an extension to our Deposit Account No. 06-0916. A duplicate copy of this paper is enclosed for use in charging the deposit account.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Date: November 21, 2001

By: _____

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PATENT
Attorney Docket No.07681.0018-01
(710/206C)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
CAMPBELL et al.	)	Group Art Unit: 1632
	)	
Serial No.: TO BE ASSIGNED	)	Examiner: D. Crouch
	)	
Filed: November 21, 2001	)	
	)	
For: UNACTIVATED OOCYTES AS CYTOPLAST RECIPIENTS FOR NUCLEAR TRANSFER	)	

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

REQUEST UNDER 37 C.F.R. § 1.607 FOR INTERFERENCE WITH U.S. PATENT 6,235,969 TO STICE ET AL.

Pursuant to the provisions of 37 C.F.R. §1.607, applicants respectfully request that an interference be declared between claim 19 in the subject application and claims 1-26 of U.S. Patent 6,235,969 to Stice et al. The patent is hereinafter referred to as "the Stice patent". A copy is attached as Exhibit A.

Applicants submit the following information in fulfillment of the requirements of 37 C.F.R. § 1.607.

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I. PROPOSED COUNT

In fulfillment of the requirement of Rule 1.607(a)(2), applicants propose the following Count for purposes of interference:

A method of producing a non-human mammalian embryo by nuclear transfer comprising:

(i) transfer of a nucleus of a non-human mammalian cell, which has passed start in the mitotic cell cycle and is in the G1 phase of the cell cycle, into an unactivated, enucleated, metaphase II-arrested oocyte of the same species as the donor cell nucleus;

(ii) activation of the recipient oocyte containing the donor cell nucleus; and

(iii) incubation of the activated oocyte to provide an embryo;

wherein the donor cell nucleus is from a mammalian differentiated cell;

or:

(i) transplantation of a non-human mammalian cell or a nucleus of a non-human mammalian cell into an enucleated oocyte of the same species as the donor cell or donor cell nucleus;

(ii) activation of the recipient oocyte containing the donor cell or donor cell nucleus; and

(iii) incubation of the activated oocyte to produce an embryo;

wherein the donor cell is a non-quiescent mammalian differentiated cell or wherein the donor nucleus is from a non-quiescent mammalian differentiated cell.

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The Proposed Count incorporates the exact language of applicants' claim 19. The Proposed Count also incorporates the exact language of claim 24 of the Stice patent.[1]

II. IDENTIFICATION OF PATENT CLAIMS CORRESPONDING TO THE PROPOSED COUNT

Claims 1-26 of the Stice patent, which are all of the claims of the patent, are directed to methods of producing non-human mammalian embryos and to methods of cloning pigs by nuclear transfer. All of the patent claims are directed to the same invention and should be designated as corresponding to the Proposed Count. *See* 37 C.F.R. § 1.606.

---

[1] An Alternative Proposed Count could read as follows:

> A method of cloning a non-human mammal by nuclear transfer comprising the method of claim 19 of Campbell et al.'s application Serial No. _____ or claim 24 of Stice et al.'s U.S. Patent No. 6,235,969.

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III. IDENTIFICATION OF APPLICANTS' CLAIMS CORRESPONDING TO THE PROPOSED COUNT

Applicants' claim 19 is also directed to methods of producing non-human mammalian embryos by nuclear transfer. This claim should be designated as corresponding to the Proposed Count.

IV. APPLICATION OF APPLICANTS' CLAIM TO THE DISCLOSURE IN THEIR APPLICATION

Applicants' claim 19 is being presented in a Preliminary Amendment filed herewith. Section (a)(5) of Rule 1.607 requires applicants to identify support in their application for any of their claims designated as corresponding to the Proposed Count.

Exhibit B annexed hereto contains each of the recitations in applicants' claim 19 and quotations from the specification supporting each recitation. Exhibit B thus satisfies the requirement of Rule 1.607(a)(5).

V. APPLICANTS ARE THE SENIOR PARTY RELATIVE TO STICE ET AL.

The Stice patent is based on a U.S. application filed July 3, 1997. The Stice patent is related to an earlier application filed on January 10, 1997. If Stice et al. can

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demonstrate that they are entitled to the benefit of the filing date of each of the earlier-filed applications as constructive reductions to practice, Stice et al.'s effective filing date for purposes of an interference would be January 10, 1997.

Applicants, on the other hand, have an effective U.S. filing date of August 31, 1995, through a series of priority applications, each of which constitutes a constructive reduction to practice of the Proposed Count. Specifically, the subject application is a continuation of parent application Serial No. 09/650,194, filed August 29, 2000, which is a continuation of grandparent application Serial No. 08/803,165, filed February 19, 1997. Thus, applicants are entitled to the benefit of the filing date of February 19, 1997, of the grandparent application because it is linked to the grandparent application through a continuation application, and thus has an identical specification to the grandparent application. 35 U.S.C. § 120.

The grandparent application, in turn, is a §371 application of PCT/GB96/02098, filed August 30, 1996. A copy of the PCT application as published under No. WO 97/07668 is attached as Exhibit C. The PCT application and the subject application are identical. Thus, applicants are also entitled to the benefit of

the filing date of August 30, 1996, of the PCT application. 35 U.S.C. § 119 and MPEP 1896.

Finally, the PCT application claims the benefit of British application No. 95 17779.6, filed August 31, 1995. A certified copy of the British priority application is of record in application Serial No. 08/803,165, filed February 19, 1997.

There are several differences between the British priority application and the subject U.S. application. These differences have been highlighted on Exhibit D, which is a copy of the subject application. It will be evident that the highlighted passages do not affect applicants' right to the benefit of the British application for the subject matter of claim 19 in the subject application. Thus, applicants are entitled to the filing date of their British priority application. 35 U.S.C. § 119.

In summary, applicants' effective filing date of August 31, 1995, can be traced from the subject continuation application through the parent and grandparent applications to the earlier PCT application and finally to the British priority application. Each of these applications constitutes a constructive reduction to practice of the Proposed Count. Because applicants' effective filing date of **August 31, 1995**, predates by **almost 17**

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months the earliest filing date of **January 10, 1997**, which Stice et al. could allege, justice requires that applicants be named the senior party in any interference that may be declared with the Stice patent.

VI. APPLICANTS AND STICE ET AL. ARE CLAIMING THE SAME PATENTABLE INVENTION

Applicants' claim 19 defines the same patentable invention as claims 1-26 in the Stice patent. Thus, interference-in-fact exists. *See* 37 C.F.R. § 1.601(j). ("An interference-in-fact exists when at least one claim of a party that is designated to correspond to a count and at least one claim of an opponent that is designated to correspond to the count define the same patentable invention.")

More particularly, 37 C.F.R. § 1.601(n) provides that one invention is "the same patentable invention" as another invention when the first invention is the same as (35 U.S.C. § 102) or is obvious (35 U.S.C. § 103) in view of the second invention, assuming the second invention is prior art with respect to the first invention.

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Recent precedent of the Trial Section of the Interference Division of the Board of Patent Appeals and Interferences indicates that resolution of whether an interference-in-fact exists involves a two-way patentability analysis. According to the Board.

> The claimed invention of Party A is presumed to be prior art vis-a-vis Party B and vice versa. The claimed invention of Party A must anticipate or render obvious the claimed invention of Party B and the claimed invention of Party B must anticipate or render obvious the claimed invention of Party A. When the two-way analysis is applied, then regardless of who ultimately prevails on the issue of priority, * * * [USPTO] assures itself that it will not issue two patents to the same patentable invention.

Winter v. Fujita, 53 U.S.P.Q.2d 1234, 1243 (Bd. Pat. App. & Intf. 1999), reh'g denied, 53 U.S.P.Q.2d 1478 (Bd. Pat. App. & Intf. 2000).

In support of their request for declaration of an interference, applicants will describe their invention and then compare the terms in Stice et al.'s claim 24 with the corresponding terms in applicants' claim 19. This comparison will convincingly show that applicants are claiming the same patentable invention as that claimed in the Stice patent and that interference-in-fact exists.

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Applicants will then show that applicants have met the one year time limit imposed by 35 U.S.C. § 135(b) by claiming this invention within one year of the issuance of the Stice patent.

A. APPLICANTS' PIONEERING WORK INVOLVING NUCLEAR TRANSFER INTO DIFFERENTIATED CELLS LEAD TO THE CLONING OF "DOLLY" THE SHEEP

The report of the cloning of "Dolly" the sheep generated enormous attention in the scientific and general press because of its novelty and the significance of the work. This cloning work is the subject of applicants' invention.

At the time of applicants' invention, animal cloning had been achieved by genetic manipulation using nuclear transfer technology: A nucleus was removed from a donor cell, then transplanted into an oocyte whose own nucleus had previously been removed. The resulting renucleated oocyte gave rise to an animal that carried the nuclear genome of only the donor of the nucleus. The individual providing the donor nucleus and the individual that developed from the renucleated oocyte were referred to as "clones".

Nuclear transfer technology first employed a donor cell that was derived from an early embryo. The cells of the embryo had not undergone substantial division and differentiation ---

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the cells were totipotent, meaning that they had the potential to develop into any type of cell in an adult.

Unlike embryo cloning, the prospect of cloning a cell from an adult seemed remote. More particularly, all animals develop from a single cell, the fertilized ovum, which gives rise to the various tissues and organs. Cells from the ovum undergo division and differentiation, which is driven by gene switching: The difference between one cell type and another is primarily in the range of genes that are active in each cell. Certain genes in the genome are "programmed" to express their proteins, leading to cell specialization at a very early stage of development within the embryo.

It was thought that a differentiated cell was committed to a specialized course of development and ultimately a specialized function. It was believed that a differentiated cell exhibited a memory for its specialized function and passed its functional characteristics on to its progeny. Prior to applicants' invention, is it was thought that once a cell became differentiated and entered a determined developmental pathway, the pathway was irreversible. No manipulation of the cell environment would, for example, cause a heart cell to differentiate into a liver cell.

Applicants' specification describes the cause of this phenomenon as follows: "During development certain genes become imprinted i.e., are altered such that they are no longer transcribed." (Specification at page 4, lines 16-18.) Applicants discovered that the "imprint" on an adult differentiated cell can indeed be removed by "reprogramming" the cell nucleus following its transfer to the enucleated, recipient oocyte. The application of this discovery produced "Dolly" the sheep in Example 2 in the subject application by nuclear transfer from an adult differentiated cell in the G_o phase of the cell cycle.

More particularly, as described in the present application, the nucleus that is transferred to the enucleated, recipient oocyte can be taken from an adult differentiated cell. "Dolly" the sheep was produced in Example 2 using a nucleus from an adult sheep cell in the G_o phase of its cell cycle. The specification teaches that an adult differentiated cell in the G_1 phase of its cell cycle could be used as well. (See pages 16-21, *infra*, for a more detailed discussion of the cell cycle.) Nuclear transfer from the adult differentiated cell into an oocyte arrested in metaphase II gave rise to a viable sheep embryo by maintaining normal ploidy (i.e. diploidy). Activating

the embryo after nuclear transfer allowed the nucleus to remain exposed to the recipient cytoplasm. This delay resulted in nuclear reprogramming so that the renucleated oocyte could be implanted in a live animal and could develop to term.

The successful cloning of "Dolly" showed, for the first time, that the nucleus from a differentiated adult cell could be reprogrammed to become totipotent once more, just like the genetic material in the fertilized oocyte from which the donor cell had ultimately developed. This successful cloning of an adult animal forced scientists to accept that genome modifications, once considered irreversible, can be reversed, and that genomes of adult cells can be reprogrammed by factors in the oocyte to make them capable once again of differentiating into any cell type.

Applicants' claim 19 is directed to a method of producing a non-human mammalian embryo by nuclear transfer using a differentiated cell from an adult donor. The resulting embryos, after implantation into a host animal, can develop into a live animal in a manner similar to the birth of "Dolly".

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B. STICE ET AL. ALSO CLAIM TO HAVE INVENTED ANIMAL CLONING BY NUCLEAR TRANSFER USING DIFFERENTIATED CELLS

The Stice patent also claims a method of cloning a non-human mammal by nuclear transfer using a differentiated cell. Stice et al. describe their work as follows:

> According to the invention, cell nuclei derived from differentiated pig cells are transplanted into enucleated pig oocytes. The nuclei are reprogrammed to direct the development of cloned embryos, which can then be transferred into recipient females to produce fetuses and offspring, or used to produce CICM cells. The cloned embryos can also be combined with fertilized embryos to produce chimeric embryos, fetuses and/or offspring.

(Exhibit A at col. 6, lines 41-47.)

The importance of differentiated cells for nuclear transfer is pointedly emphasized in the Stice patent: "Again the present invention is novel because differentiated cell types are used." (Exhibit A at col. 9, lines 18-19.)

The use of "differentiated cells" was characterized as "novel" and the essence of their work. Stice et al. were apparently unaware of applicants' work using differentiated cells in nuclear transfer when Stice et al. filed their application.

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C. APPLICANTS' CLAIMED INVENTION IS THE SAME AS THE SUBJECT MATTER OF THE STICE ET AL. AS SHOWN BY A COMPARISON OF APPLICANTS' CLAIM 19 WITH CLAIM 24 OF THE STICE PATENT.

Table 2 and the comments that follow show that applicants' claim 19 contains limitations that are the same as limitations in claim 24 of the Stice patent. These are the two claims that comprise applicants' Proposed Count on pages 2-3, *supra*.

TABLE 2

COMPARISON OF APPLICANTS' CLAIM 19 WITH CLAIM 24 OF THE STICE PATENT.

Applicants' claim 19	Claim 24 of the Stice patent
19. A method of producing	19. A method of producing
a non-human mammalian embryo	a non-human mammalian embryo
by nuclear transfer	by nuclear transfer
comprising:	comprising:
(i) transfer	(i) transplantation
of a nucleus	of a . . . nucleus
of a non-human	of a non-human
mammalian cell,	mammalian cell
which has passed start in the mitotic cell cycle and is in the G1 phase of the cell cycle,	
into an	into an
unactivated,	
enucleated,	enucleated
metaphase II-arrested	
oocyte	oocyte
of the same species as the donor cell nucleus;	of the same species as the . . . donor cell nucleus[2],
(ii) activation	activation

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Applicants' claim 19	Claim 24 of the Stice patent
of the recipient oocyte containing the donor cell nucleus; and	of the recipient oocyte containing the . . . donor cell nucleus; and
(iii) incubation	incubation
of the activated oocyte	of the activated oocyte
to provide an embryo;	to produce an embryo;
wherein the donor cell nucleus	wherein . . . the donor nucleus[3]
is from a	is from a
	non-quiescent
mammalian	mammalian
differentiated cell.	differentiated cell.

[2] The complete clause of Stice claim 24 reads as follows: "transplantation of a non-human mammalian cell or a nucleus of a non-human mammalian cell into an enucleated oocyte of the same species as the donor cell or donor cell nucleus. . . ." (Exhibit A at col. 20, lines 53-56)

[3] The complete clause in Stice claim 24 reads as follows: "wherein the donor cell is a non-quiescent mammalian differentiated cell or wherein the donor nucleus is from a non-quiescent mammalian differentiated cell." (Exhibit A at col. 20, lines 59-61.)

It will be evident from Table 2 that applicants' claim 19 contains recitations that are identical to recitations in claim 24 of the Stice patent. As described in Exhibit B attached hereto, all of these recitations are supported by applicants' specification. Support for these recitations in applicants claim will not be further discussed.

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Instead, the terms in applicants' claim that are absent from Stice et al.'s claim, or appear to be different, will now be discussed. These terms are arranged below in a different order than they appear in Table 2 to facilitate an understanding of the meaning of the terms and their relation to each other. This discussion will leave no doubt that applicants and Stice et al. are claiming the same invention and that interference-in-fact exists.

(1) The recitation of a nuclear donor cell "which has passed start in the mitotic cell cycle and is in the G1 phase of the cell cycle" in applicants' claim

vs.

the recitation of a "non-quiescent" cell in Stice et al.'s claim

The nucleus for cloning the non-human mammal is taken from a particular type of non-human mammalian cell, which is referred to herein as the "nuclear donor" for the sake of brevity. Specifically, in applicants' claim 19, the nuclear donor is a cell "which has passed start in the mitotic cell cycle and is in the G1 phase of the cell cycle." Stice et al.'s nuclear donor is a cell that is "non-quiescent." The use of these terms does not impart separate patentability to either claim. Each term describes a cell that is actively dividing.

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The prosecution history of related Stice U.S. Patent 6,215,041 makes it clear that Stice et al. understood that a "non-quiescent" cell is an "actively dividing" cell. Dr. James M. Robl, one of the Stice et al. inventors, stated that:

> (7) In particular, the novel developments discovered by the inventors of this application include:
>
> (i) the successful use of cells committed to a somatic cell lineage for nuclear transfer or transplantation;
>
> (ii) the successful use of actively dividing, i.e., non-quiescent cells for nuclear transplantation; and
>
> (iii) the use of somatic cell genetic modification to produce genetically modified animals.

(Exhibit E, Declaration of James M. Robl, Ph.D. Pursuant To 37 C.F.R. §1.132 at page 3; original emphasis.)

Just as the "non-quiescent" nuclear donor cell recited in Stice et al.'s claim is "actively dividing", applicants' nuclear donor cell "which has passed start in the mitotic cell cycle and is in the G1 phase of the cell cycle" is "actively dividing." Underlying the meaning of a nuclear donor cell "which has passed start in the mitotic cell cycle and is in the G1 phase of the cell cycle in applicants' claim is an understanding of the cell cycle and an appreciation for the limited number of phases in

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the cycle. The mitotic cell cycle is described in applicants' specification as follows.

The mitotic cell cycle has four distinct phases, G1, S, G2, and M. The beginning event in the cell cycle, called start, takes place in the G1 phase and has a unique function. The decision or commitment to undergo another cell cycle is made at start. Once a cell has passed through start, it passes through the remainder of the G1 phase, which is the pre-DNA synthesis phase.

The second stage, the S phase, is when DNA synthesis takes place. This is followed by the G2 phase, which is the period between DNA synthesis and mitosis. Mitosis itself occurs at the M phase. Quiescent cells (which include cells in which quiescence has been induced as well as those cells which are naturally quiescent, such as certain fully differentiated cells) are generally regarded as not being in any of these four phases of the cycle; they are usually described as being in a G0 state, so as to indicate that they would not normally progress through the cycle. (Applicants' specification at page 7, line 26 to page 8, line 11.) It is evident from this description that there are only four phases in the mitotic cell cycle, namely, the G1, S, G2, and M phases.

Once a cell enters the mitotic cell cycle by passing through "start", the cell is committed to pass through the remainder of the G1 phase of the cell cycle in which the cell is actively dividing. These are the conditions recited in applicants' claim 19. This claim recites that the nuclear donor is a cell "which has passed start in the mitotic cell cycle and is in the G1 phase of the cell cycle."

Instead of reciting that the nuclear donor is a cell "which has passed start in the mitotic cell cycle and is in the G1 phase of the cell cycle," Stice et al.'s claim 24 recites that the donor cell is "non-quiescent". By not indicating which stage of the cell cycle the "non-quiescent" cell is in, claim 24 of the Stice patent reads on a nuclear donor cell in any phase of the cell cycle, namely, the G1, S, G2, or M phase. Thus, the difference between the two terms is that applicants' claim reads on the use of a nuclear donor cell actively dividing in the G1 phase of the cell cycle, whereas Stice et al.'s claim reads on a nuclear donor cell actively dividing in any one of the four phases of the cell cycle, including the G1 phase.

Applying the analysis required by the *Winter v. Fujita* case, applicants' species of an actively dividing nuclear donor cell "which has passed start in the mitotic cell cycle and is in

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the G1 phase of the cell cycle" anticipates the Stice et al. claim embracing a "non-quiescent" nuclear donor cell that is actively dividing in any one of the four phases of the cell cycle, assuming applicants' claim is prior art to Stice et al.'s claim and all of the other claim limitations are the same. A later genus claim is never patentable over an earlier species claim. *Eli Lilly v. Barr Laboratories, Inc.*, 222 F.3d 973, 976 (Fed. Cir. 2000).

Following a similar analysis, but assuming the Stice et al. claim is prior art to applicants' claim, applicants' claim to the use of an actively dividing nuclear donor cell "which has passed start in the mitotic cell cycle and is in the G1 phase of the cell cycle" would be rendered *prima facie* obvious by Stice et al.'s claim encompassing the use of an actively dividing nuclear donor cell in one of the four phases of the cell cycle. Indeed, applicants' claim to one phase of the cell cycle may be anticipated by Stice et al.'s claim embracing a nuclear donor cell in any one of the four phases, one of which is recited in applicants' claim, because it is well established that a small genus can anticipate a species within that genus. *See, e.g., In re Petering*, 301 F.2d 676, 682, 133 U.S.P.Q. 275, 280 (C.C.P.A. 1962) (Genus of 20 compounds describes each species within the

meaning of § 102(b)); *In re Schaumann*, 572 F.2d 312, 316-317, 197 U.S.P.Q. 5, 9 (C.C.P.A. 1978) (Prior art disclosure embraces such a limited number of compounds closely related to one another in structure that it "provides a description of those compounds just as surely as if they were identified in the reference by name.").

In any event, the recitation of a donor cell "which has passed start in the mitotic cell cycle and is in the G1 phase of the cell cycle" in applicants' claim and the recitation of a donor cell that is "non-quiescent" in Stice et al.'s claim do not impart patentable distinctness to either claim. Both types of donor cells are "actively dividing." Thus, one claim anticipates the other claim, while the other claim at the least renders the first claim *prima facie* obvious.

(2) The recitation of "metaphase II - arrested" in applicants' claim

Applicants' claim contains another recitation that is absent from Stice et al.'s claims, namely, that the oocyte into which the nucleus from the nuclear donor is transferred is in a particular phase of its cell cycle. It is "metaphase II-arrested." The absence of this term from Stice et al.'s claim is immaterial for determining "same patentable invention".

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Stice et al. teach in their specification that metaphase-II oocytes should be used for successful nuclear transfer. Specifically, Stice et al. state that:

> Additionally, metaphase II stage oocytes, which have been matured in vivo have been successfully used in nuclear transfer techniques.
>
> * * *
>
> The stage of maturation of the oocyte at nucleation and nuclear transfer has been reported to be significant to the success of NT methods. (See e.g., Prather et al., *Differentiation*, 48, 1-8, 1991). In general, successful mammalian embryo cloning practices use the metaphase II stage oocyte as the recipient oocyte because at this stage it is believed that the oocyte can be or is sufficiently "activated" to treat the introduced nucleus as it does a fertilizing sperm.

(Exhibit A at col. 9, lines 41-56.)

According to Stice et al., oocytes in metaphase II are the cells of choice to ensure successful nuclear transfer. Moreover, Stice et al. indicate this was known in the art.

The identification of a "metaphase II-arrested" oocyte as the recipient of the nucleus from the non-quiescent differentiated cell in the G1 phase of the cell cycle in applicants' claim and the absence of this recitation from Stice et al.'s claim does not impart separate patentability to either claim applying the analysis under *Winter v. Fujita*. Specifically, assuming applicants' claim is prior art to Stice

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et al.'s claim and all of the other claim limitations are the same, applicants' claim would anticipate the claim of Stice et al.; applicants' claim would contain all of the limitations of the claim of Stice et al., and the additional limitation "metaphase II-arrested" oocyte in applicants' claim would not change the analysis.

Applying the test in reverse, and assuming the Stice et al. claim is prior art to applicants' claim and that all of the other limitations are the same, the recitation of a "metaphase II-arrested" oocyte in applicants' claim would have been obvious in view of the Stice et al. claim taken in view of the knowledge in the art that a metaphase II oocyte was the cell of choice for nuclear transfer.

(3) The recitation of "unactivated" in applicants' claim

Following transfer of the nucleus into the enucleated oocyte and reprogramming of the genes of the donor nucleus, the resulting renucleated oocyte is activated to resume embryonic development. Applicants and Stice et al. each require a step of "activating" the resulting reconstructed embryo or NT unit in their claims.

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Applicants' claim also recites that the enucleated oocyte is "unactivated" at the time of the nucleus is transferred from the nuclear donor. While the term "unactivated" is not recited in Stice et al.'s claim, it is inherent in the claim as the claim requires "activation of the recipient oocyte." If the oocyte had already been activated, this activation step would be unnecessary.

In addition, if the oocyte had already been activated, the claim of Stice et al. should have included a step of interrupting activation in order to give meaning to the subsequent step of "activation of" the resultant renucleated oocyte. The Stice et al. claim does not include a step of interrupting activation of an activated oocyte, and accordingly, the only reasonable interpretation of the claim is that the oocyte is "unactivated" when the nucleus is transferred.

The term "unactivated" oocyte, which is inherent in Stice et al.'s claim, does not patentably distinguish the claim from applicants' claim, or vice versa.

In summary, the comparison of applicants' claim 19 with claim 24 of the Stice et al. patent shows that most of the claim limitations are identical and those that are not do not impart separate patentability to either claim. The only conclusion is

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that these two claims define the same patentable invention and that interference-in-fact exists.

D. APPLICANTS HAVE MET THE ONE YEAR TIME LIMIT IMPOSED BY 35 U.S.C. § 135(b) BY CLAIMING THE SAME PATENTABLE INVENTION AS STICE ET AL. WITHIN ONE YEAR AFTER THE STICE PATENT ISSUED

The Stice et al. patent issued on **May 22, 2001.** Applicants are presenting claim 19 in the subject application in the Preliminary Amendment filed herewith. Applicants thus claimed the interfering subject matter within one year after the Stice patent issued, thereby meeting the one-year time limit imposed by 35 U.S.C. § 135(b).

VII. CONCLUSION

It is a fundamental principle that issuance of two patents for inventions that are either identical to or not patentably distinct from each other must be avoided. M.P.E.P. 2306, *citing Aelony v. Arni*, 547 F.2d 566, 192 U.S.P.Q. 486 (C.C.P.A. 1997). This mandate has a matter of urgency attached to it in the present case in which a patent has already been issued to an entity that would be the junior party in an interference with applicants. An interference should be declared, and applicants should be designated as the senior party in the interference.

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If there are any fees due in connection with the filing of this Request, please charge such fees to our Deposit Account No. 06-0916.

Respectfully submitted,
FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Date: November 21, 2001

By: 

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PATENT
Customer No. 22,852
Attorney Docket No. 07681.0018-01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Campbell et al.	)	Group Art Unit: 1633 (Prior Appln.)
	)	
Serial No.: Unknown	)	Examiner: D. Crouch (Prior Appln.)
(Prior Appl. Ser. No. 09/650,285)	)	
	)	
Filed: November 21, 2001	)	

For: UNACTIVATED OOCYTES AS CYTOPLAST RECIPIENTS FOR NUCLEAR TRANSFER

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

SUBMISSION OF FORMAL DRAWINGS

Subject to the approval of the Examiner, applicants herewith submit 1 sheet of Formal Drawings (Figure 1). If the Formal Drawings for any reason are not in full compliance with the pertinent statutes and regulations, please so advise the undersigned.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Date: November 21, 2001

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Patent
Attorney's Docket No. 000270-007

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

COPY

In re Patent Application of	)	
	)	
Steven L. STICE et al	)	Group Art Unit: 1819
	)	
Serial No.: 08/781,752	)	Examiner: D. Crouch
	)	
Filed: January 10, 1997	)	
	)	
For: CLONING USING DONOR NUCLEI FROM DIFFERENTIATED FETAL AND ADULT CELLS	)	

DECLARATION OF JAMES M. ROBL Ph.D. PURSUANT TO 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, James M. Robl, Ph.D., declare and state as follows:

(1) I reside at 196 Old Enfield, Belchertown, Massachusetts 01007;

(2) I am a Professor in the Veterinary & Animal Sciences Department at the University of Massachusetts at Amherst Massachusetts;

(3) I have substantial knowledge and expertise in the areas of cloning and transgenic animals. My expertise is substantiated by the attached curriculum vitae.

(4) Based on such expertise, I am frequently asked to give presentations and talks relating to cloning and transgenic animals.

(5) I have reviewed the most recent Office Action issued on June 22, 1998, in the above-identified application. In particular, I have carefully reviewed the enablement rejection that bridges pages 5 to 8 of the Office Action. Based on such review, it is my understanding that the Examiner apparently is of the opinion that the subject application only enables a method for cloning a bovine comprising the specific steps of:

(i) inserting the fibroblast or nucleus isolated from a 45 day bovine fetus into the perivitelline space of a bovine oocyte matured *in vitro* to metaphase II;

(ii) fusing the oocyte and fibroblast or nucleus to form a NT unit; activating the NT unit by incubating such NT unit for 26-27 hours post-maturation in media comprising 5 um ionomycin and 2mM DMAP for 4 minutes;

(iii) culturing the NT units in CR1aa-2mM DMAP media for 4-5 hours;

(iv) and culturing the activated NT units in CR1aa media containing mouse fibroblast feeder cells for 5-8 days after activation and transforming to a host bovine for development into a fetus; as well as offspring and progeny produced by such methods; and producing a bovine CICM cell by the same method except that the transfer to a host bovine is omitted and the cultured activated NT units are

desegregated to produce the inner cell mass of embryos. Based on the following, I respectfully disagree with the Examiner's conclusion.

(6) However, prior to specifically addressing the enablement concerns raised in the office Action, I will summarize some of the novel discoveries which form the basis of the subject invention. It is believed that this discussion will clarify why I am of the opinion that the claims are commensurate in scope with the subject disclosure, especially given the truly pioneering nature of the present invention.

(7) In particular, the novel developments discovered by the inventors of this application include:

i) the successful use of cells committed to a somatic cell lineage for nuclear transfer or transplantation;

ii) the successful use of actively dividing, i.e., non-quiescent cells for nuclear transplantation; and

iii) the use of somatic cell genetic modification to produce genetically modified animals.

Prior to the filing date of this application, to the best of my knowledge, there had been no previous report of the use of cells committed to a somatic cell lineage for successful nuclear transplantation, i.e., that gave rise to viable offspring. I believe this

to be a novel and surprising discovery based on the history of nuclear transplantation prior to the present invention.

(8) In this regard, I note that nuclear transfer first gained acceptance in the 1960's with amphibian nuclear transplantation. (Diberardino, M.A. 1980, "Genetic stability and modulation of metazoan nuclei transplanted into eggs and ooctyes", *Differentiation*, 17-17-30; Diberardino, M.A., N.J. Hoffner and L.D. Etkin, 1984; "Activation of dormant genes in specialized cells", *Science,* 224:946-952; Prather, R.S. and Robl, J. M., 1991, "Cloning by nuclear transfer and splitting in laboratory and domestic animal embryos", In: *Animal Applications of Research in Mammalian Development*, R.A. Pederson, A. McLaren and N. First (ed.), Spring Harbor Laboratory Press.) Nuclear transfer was initially conducted in amphibians in part because of the relatively large size of the amphibian oocyte relative to that of mammals. The results of these experiments indicated to those skilled in the art that the degree of differentiation of the donor nucleus was greatly instrumental, if not determinative, as to whether a recipient oocyte containing such cell or nucleus could effectively reprogram said nucleus and produce a viable embryo. (Diberardino, M.A., N.J. Hoffner and L.D. Etkin, 1984, "Activation of dormant genes in specialized cells.", *Scienc,e* 224:946-952; Prather, R.S. and Robl, J. M.,1991, "Cloning by nuclear transfer and splitting in laboratory and domestic animal embryos", In: *Animal Applications of*

Research in Mammalian Development, R.A. Pederson, A. McLaren and N. First (ed.), Spring Harbor Laboratory Press) This work is well documented and was considered dogma prior to the filing of this application.

(9) Much later, in the mid 1980's, after microsurgical techniques had been perfected, researchers, including myself, investigated whether nuclear transfer could be extrapolated to mammals. I worked out the first procedures for cloning cattle (Robl, J. M., R. Prather, F. Barnes, W. Eyestone, D. Northey, B. Gilligan and N.L. First, 1987, "Nuclear transplantation in bovine embryos", *J. Anim. Sci.*, 64:642-647) and my lab was the first to clone a rabbit by nuclear transfer using donor nuclei from earlier embryonic cells (Stice, S.L. and Robl, J. M., 1988, "Nuclear reprogramming in nuclear transplant rabbit embryos", *Biol. Reprod.*, 39:657-664). Also, using similar techniques, bovines (Prather, R.S., FL. Barnes, ML. Sims, Robl, J. M., W.H. Eyestone and N.L. First, 1987, "Nuclear transplantation in the bovine embryo: assessment of donor nuclei and recipient oocyte", *Biol. Reprod.*, 37:859-866) and sheep (Willadsen, S.M., 1986, "Nuclear transplantation in sheep embryos", *Nature*, (Lond) 320:63-65), and putatively porcines (however, this work apparently has never been reproduced) (Prather, R.S., M.M. Sims and N.L. First, 1989, "Nuclear transplantation in pig embryos", *Biol. Reprod.*, 41:414), were cloned by the transplantation of the cell or nucleus of very early embryos into enucleated oocytes.

(10) Moreover, work in our laboratory, and others, investigated the possibility of producing nuclear transfer embryos with donor nuclei obtained from progressively more differentiated cells. However, our results and those obtained by other groups, indicated that when the embryo progresses to the blastocyst stage (the embryonic stage where the first two cell lineages separate) that the efficiency of nuclear transfer decreases dramatically (Collas, P. and J.M. Robl, 1991, "Relationship between nuclear remodeling and development in nuclear transplant rabbit embryos", *Biol. Reprod.*, 45:455-465). For example, it was found that trophectodermal cells (the cells that form the placenta) did not support development of the nuclear fusion to the blastocyst stage. (Collas, P. and J.M. Robl, 1991, "Relationship between nuclear remodeling and development in nuclear transplant rabbit embryos", *Biol. Reprod.*, 45:455-465) By contrast, inner cell mass cells (cells which form both somatic and germ cells) were found to support a low rate of development to the blastocyst stage with some offspring obtained. (Collas P, Barnes FL, "Nuclear transplantation by microinjection of inner cell mass and granulosa cell nuclei", *Mol Reprod Devel.*, 1994, 38:264-267) Moreover, further work suggested that inner cell mass cells which were cultured for a short period of time could support the development to term. (Sims M, First NL, "Production of calves by transfer of nuclei from cultured inner cell mass cells", *Proc Natl Acad Sci* ,1994, 91:6143-6147)

(11) Based on these results, and that of other researchers, it was the overwhelming opinion of those skilled in the art at that time, including myself, that observations made with amphibian nuclear transfer experiments would likely be observed in mammals. That is to say, it was widely thought by researchers working in the area of cloning prior to the present invention that once a cell becomes committed to a particular somatic cell lineage that its nucleus irreversibly loses its ability to become "reprogrammed", i.e., to support full term development when used as a nuclear donor for nuclear transfer. While the exact molecular explanation for the apparent inability of somatic cells to be effectively reprogrammed was unknown, it was hypothesized to be the result of changes in DNA methylation, histone acetylation and factors controlling transitions in chromatin structure that occur during cell differentiation. Moreover, it was believed that these cellular changes could not be reversed.

(12) The discovery made by the present inventors, and subsequently reported by the Roslin Institute after the filing date of this application, i.e., that cells committed to somatic cell lineage could support development when used as nuclear transfer donors, is actually the culmination of a progression of experiments and observations made by our laboratory. For example, we demonstrated in 1990 (unpublished observations, Collas, P. and J.M. Robl, 1991, "Relationship between nuclear

remodeling and development in nuclear transplant rabbit embryos", *Biol. Reprod.*, 45:455-465; Collas P, Barnes FL, "Nuclear transplantation by microinjection of inner cell mass and granulosa cell nuclei", *Mol Reprod Devel*, 1994, 38:264-267) that somatic cells could support development to the blastocyst stage, but not beyond. This seemingly confirmed the general view held by those skilled in the cloning art at that time concerning the irreversible changes to cells that occur during differentiation. In fact, because of this erroneous belief, our first work with somatic cell nuclear transplantation was not conducted with the goal of producing full term offspring. Our laboratory, like others at that time, was of the opinion that this would not be feasible. Rather, we were interested in producing blastocysts by somatic cell nuclear transplantation, and using the resultant blastocyst stage embryos to produce ES-like cells. The thought was that somatic cell-derived ES-like cells might be able to contribute to the development of fully differentiated tissues if grown in association with normal cells in a chimera. This work was successful. (Cibelli, J.B., S.L. Stice, P.J. Golueke, J.J. Kane, J. Jerry, C. Blackwell, F.A. Ponce de Leon and Robl, J.M., 1998, "Transgenic bovine chimeric offspring produced from somatic cell-derived stem-like cells", *Nature/Biotechnology*,16: 642-646)

(13) Based on the surprising success of these experiments, we tried to determine whether somatic cell nuclear transplant embryos could support early

development on their own *in vivo* and provide a source of fetal tissue. These experiments were also surprisingly effective and resulted in healthy 40 day fetuses. (Zwada, M.W., J.B. Cibelli, PK. Choi, E.D. Clarkson, P.J. Golueke, S.E. Witta, K.P. Bell, J. Kane, F.A. Ponce de Leon, D.J. Jerry, Robl, J.M., C.R. Freed and S.L. Stice, 1998, "Somatic cell cloning-produced transgenic bovine neurons for transplantation in parkinsonian rats", *Nature Medicine*, 4:569-574)

(14) In the course of these studies we also determined whether these fetal cells could be genetically manipulated *in vitro*, i.e., by the introduction of a heterologous DNA by electroporation, prior to their use as nuclear transplant donors. These experiments were effected because an important goal of cloning, if not the most important goal of cloning, is to provide a reproducible source of cells having a desired genotype, e.g., which express a particular transgene. Thereby, the resultant cloned embryos or animals can be used to produce a desired gene product or for cellular transplantation therapies. However, it was by no means predictable that these experiments would be successful. To the contrary, I am of the opinion, based on the state of the art at the time of the invention, that the prevailing expectation would have been that these cells, given their differentiated state, coupled with the fact that they were manipulated in tissue culture and then transfected with a foreign DNA, would either not give rise to nuclear transfer embryos at all, or would produce embryos that

would only differentiate to early stages. However, as can be seen from the experiments, the results of which are reported in this application, quite surprisingly, these cells when inserted in enucleated bovine oocytes gave rise to apparently perfectly healthy 40 day transgenic bovine embryos.

(15) Thereupon, based on the surprising success of the above-described experiments, we then attempted to determine whether somatic cell nuclear transplant embryos would give rise to viable full-term bovine offspring, and more desirably, transgenic viable full-term bovine offspring. As the Examiner is aware, and has been well reported in the press, it was astoundingly discovered that cells which are committed to a differentiated cell type, which cells were moreover made transgenic (transgenic fetal fibroblasts) when used as nuclear transfer donors, gave rise to healthy, transgenic bovine offspring. Moreover, these results have been successfully repeated by us and other groups. In fact, based on the reproducibility and efficiency of the subject cloning technique, the licensee of this application has entered into a collaboration with Genzyme Transgenics Corporation to make cloned transgenic bovines that produce a polypeptide (HSA) in their milk. It is further noted that using the basic cloning methods which are the subject of this application, a transgenic bovine that contains the HSA gene has been successfully obtained. With this basic understanding of the state of the nuclear transfer art that existed prior to the present

invention, and the developments and discoveries that culminated in the subject invention, I will now address the various enablement concerns raised by the Examiner.

(16) Types and Age of Differentiated Cells Used for Nuclear Transfer

As discussed, our invention involves the generic discovery that cells committed to a somatic cell lineage, which optionally are transgenic, can be used as nuclear transfer donors to produce viable fetuses and offspring. Contrary to the Examiner's position, the efficacy of the invention does not require that such somatic cells be fibroblasts isolated from 45 day old bovine offspring. In fact, our results and those of others supports our claim that a wide variety of types and ages of cells committed to a somatic cell lineage can be used successfully for nuclear transfer. Moreover, our results and those of others further support the view that these cells may be used to produce cloned transgenic animals.

(17) For example, two calves have been produced at the Ishikawa Prefecture Livestock Research Centre in Japan from oviduct cells collected from a cow at slaughter. (Hadfield, P. and A. Coghlan, "Permature birth repeats the Dolly mixture", *New Scientist*, July 11, 1998) Also, Jean-Paul Renard from INRA in France has produced a calf from muscle cells from a fetus. (MacKenzie, D. and P. Cohen, 1998., "A French calf answers some of the questions about cloning", *New Scientist*, March 21) Further, David Wells from New Zealand has produced a calf from fibroblast cells

from an adult cow. (Wells, D.N., 1998, "Cloning symposium: Reprogramming Cell Fate - Transgenesis and Cloning", Monash Medical Center, Melbourne, Australia, April 15-16)

(18) Moreover, our experiments to date indicate that cells obtained from fetuses, calves, young adults and aged adults all can be grown in culture and can be used as nuclear donor cells to produce cloned animals. However, it should be noted that the length of the cell cycle and the life-span of the cells shortens with the age of the donor. Furthermore, the percentage of healthy embryos produced from adult cells decreases with the age of the animal. This suggests that actively dividing cells can more readily support development following nuclear transfer than cells progressing towards a quiescent state. However, it does not support a proposition that only cells of a certain age can be used as suitable nuclear transfer donors.

(19) Interestingly, there is considerable variation in the length of the cell cycle within these populations. In fetal populations, some cells divide as rapidly as every 12 hours with most having divided by 24 hours. By contrast, in cell populations obtained from adult cells, a few cells will divide relatively quickly, but most require more than 24 hours to divide. These results explain why adults can be cloned, but the efficiency appears to be much lower than with fetuses. These observations also lend credence to the observation made by us, namely that actively dividing cells are capable

of being used as donor nuclei, or cells, during nuclear transfer to produce viable embryos.

This is contrary to the work of the Roslin Institute (published after the filing date of this application), which instead reported the use of quiescent, i.e., non-actively dividing cells, to produce cloned sheep. (Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS, "Viable offspring derived from fetal and adult mammalian cells", *Nature,* 1997, 1385:810-813) In fact, this significant difference in our cloning method *vis-a-vis* that reported by the Roslin Institute was discussed by us in a letter to the Editors of *Science*, 281:1611 (1998) (a copy of which is attached to this Declaration). In my opinion, this difference explains why the cloning methods reported in this application are highly reproducible.

(20) Non-Criticality of *In vitro* Maturation

The Examiner has seemingly concluded that the use of *in vitro* matured oocytes is critical to the efficacy of the invention. This, however, is not correct. While the Examiner is correct in the fact that the use of *in vitro* matured oocytes is exemplified in the actual working examples, this is not essential to the efficacy of the subject cloning methods. *In vitro* matured oocytes were used largely because of supply and cost concerns. Essentially, immature oocytes, rather than *in vivo* matured oocytes were used because we did not have access to *in vivo* matured oocytes and because *in vitro*

mature oocytes can be obtained in plentiful numbers (e.g., from slaughterhouse suppliers). However, as stated in our patent application, *in vivo* matured oocytes can also be used for nuclear transfer. For example, *in vivo* matured oocytes can be collected from either non-superovulated or ovulated cows or heifers and used for enucleation. In fact, *in vivo* oocytes have recently been used by us for cloning and a calf was successfully produced this month. Therefore, it is clear that the efficacy of the subject cloning method does not rely on a particular *in vitro* matured procedure, or even that *in vitro* matured oocytes be used at all.

(21) Oocyte Activation Protocol

Also, contrary to the Office Action, the efficacy of the subject cloning methods is not limited to the specific activation conditions exemplified in the working examples. As explained above, the truly novel discovery made by us are that (1) cells committed to a somatic cell lineage can support development to term following nuclear transfer; (2) the use of actively dividing, non-quiescent, cells for nuclear transplantation, and (3) the use of somatic cell genetic modification to produce genetically modified animals. The specific activation protocol that is used was not critical to the cloning method.

(22) With respect thereto, my laboratory has studied the activation process in great detail over the past twelve years. Activation is a process that involves the

elevation of intracellular calcium in the egg. The sperm normally produces oscillations in calcium concentration that last for several hours. Artificial activation protocols have been used on eggs for many years. Early work indicated that ethanol, electrical shock, cooling, calcium-free media, various anesthetics and a variety of other stimuli could cause activation. (Whittingham, D.G., 1980, "Parthenogenesis in mammals", *Oxford Rev. Reprod, Biol.*, 2:205-231) In more recent years, with the development of procedures for measuring intracellular calcium and the various intracellular responses to calcium, more specific approaches have been developed. For example, we now know that electrical pulses cause transient increases in intracellular calcium by inducing pores in the membrane and allowing calcium to flood into the cell from the extracellular media. (Fissore, R.A. and Robl, J.M., 1992, "Intracellular calcium response of rabbit oocytes to electrical stimulation", *Mol. Reprod. Devel.*, 32:9-16; Collas, P., J.J. Balise, G.A. Hofman and Robl, J.M., 1989, "Electrical activation of mouse oocytes", *Theriogenology*, 32:835-844; Robl, J.M., P. Collas, R. Fissore and JR. Dobrinsky, 1992, "Electrically induced fusion and activation in nuclear transplant embryos", In: *Guide to Electroporation and Electrofusion*; D. Chang, B.M. Chassy, J.A. Saunders and A.E. Sowers (ed.), Academic Press, Inc., San Diego, CA; Collas, P., R. Fissore, J. M. Robl, E.J. Sullivan and F.L. Barnes, 1993, "Electrically-induced calcium elevation, activation and parthenogenetic development of bovine oocytes", *Mol. Reprod. Devel.*,

34:212-223; Collas, P., R. Fissore and J.M. Robl, 1993, "Preparation of nuclear transplant embryos by electroporation", *Anal. Biochem.*, 208:1-9) Multiple pulses can be used to duplicate sperm-induced calcium oscillations. Injection of such intracellular second messengers such as IP3, or its long acting analogues, GTP, or its long acting analogues, or calcium itself can duplicate sperm-induced calcium rises. Other compounds that cause calcium rises, although less physiological, are ethanol and calcium ionophores. (Fissore, R.A. and Robl, J.M., 1993, "Sperm, inositol trisphosphate and thimerosal induced intracellular Ca2+ elevations in rabbit eggs", *Devel. Biol.*, 159:122-130; Fissore, R.A. and Robl, J.M., 1994, "Mechanism of calcium oscillations in fertilized rabbits eggs", *Devel. Biol.*, 166:634-642; Fissore, R.A., Pinto-Correia, C. and J.M. Robl, 1995, "Inositol trisphosphate-induced calcium release in the generation of calcium oscillations in bovine eggs", *Biol Reprod.*, 53:766-774; Collas, P., Chang, T., Long, C. and J.M. Robl, 1995, "Inactivation of histone H1 kinase by Ca^{2+} in rabbit oocytes", *Mol. Reprod. Devel.*, 40:253-258.) The second part of the activation event is a decrease in a cell cycle regulatory kinase called MPF. This results in a decrease in the phosphorylation of many different proteins in the cell and the progression to interphase. This part of the process can be duplicated by various kinase inhibitors. MPF can be inactivated directly by inhibiting protein synthesis and compounds like puromycin and cycloheximide have

been used successfully in oocyte activation protocols. Currently, there are a number of different combinations of the above that are being used successfully for oocyte activation in various laboratories around the world. Our use of the ionomycin/DMAP procedure was more a matter of convenience than a view that it was better than various other protocols, and it is certainly not critical for the success of the procedure.

For the Examiner's convenience, a brief overview of different activation procedures known prior to the filing of this application are summarized below:

Cell type	Activation	Culture media	Reference
Blastomeres	Electrical	B2 + oviductal cells	(Ectors et al. 1995)
Blastomeres	Electrical	CRI	(Zakhartchenko et al. 1995)
Blastomeres	Electrical	Bovine oviduct epithelial cells	(Campbell et al. 1993)
Blastomeres	Electrical	Modified Brinsters Ovum Culture Medium	(Barnes et al. 1993)
Blastomeres	Chilling	Sheep oviduct	(Westhusin et al. 1996)
ICM cells	Chilling	CRI + MEM + BME	(Keefer et al. 1994)
Oogonia	Ion+DMAP	TCM 199 + Steer serum	(Lavoir et al. 1997)
Blatomeres	Electrical	TCM 199 + calf serum	(Takano H. 1996)

Barnes, F. L., Collas, P., Powell, R., King, W. A., Westhusin, M., and Shepherd, D. (1993). "Influence of Recipient Oocyte Cell Cycle Stage on DNA Synthesis, Nuclear Envelope Breakdown, Chromosome Constitution, and Development in Nuclear Transplant Bovine Embryos." *Molecular Reproduction and Development*(36), 33-41.

Campbell, K. H. S., Ritchie, W. A., and Wilmut, I. (1993). "Nuclear-Cytoplasmic Interactions during the First Cell Cycle of Nuclear Transfer Reconstructed Bovine Embryos: Implications for Deoxyribonucleic Acid Replication and Development." *Biology of Reproduction*(49), 933-942.

Ectors, F.J., Delval, A., Smith, L. C., Touati, K., Remy, B., Beckers, J.-F., and Ectors, F. (1995). "Viability of Cloned Bovine Embryos After One or Two Cycles of Nuclear Transfer and In Vitro Culture." *Theriogenology*(44), 925-933.

Keefer, C. L., Stice, S. L., and Matthews, D. L. (I 994). "Bovine Inner Cell Mass Cells as Donor Nuclei in the Production of Nuclear Transfer Embryos and Calves." *Biology of Reproduction*(50), 935-939.

Lavoir, M.-C., Rumph, N., Moens, A., King, W. A., Plante, Y., Johnson, W. H., Ding, J., and Betteridge, K. J. (I 997). "Development of Bovine Nuclear Transfer Embryos Made with Oogonia." *Biology of Reproduction*(56), 194-199.

Takano H., K. C., Kato Y, Tsunoda Y. (I 996). "Cloning of bovine embryos by multiple nuclear transfer." *Theriogenology*, 47, 1365-1373.

Westhusin, M. E., Collas, P., Marek, D., Sullivan, E., Stepp, P., Pryor, J., and Barnes, F. (1996). "Reducing the Amount of Cytoplasm Available for Early Embryonic Development Decreases the Quality But Not Quantity of Embryos Produced by In Vitro Fertilization and Nuclear Transplantation." *Theriogenology*(46), 243-252.

Zakhartchenko, V., Wolf, E., Palma, G. A., and Brem, G. (I 995). "Effect of Donor Embryo Cell Number and Cell Size on the Efficiency of Bovine Embyro Cloning." *Molecular Reproduction and Development*(42), 53-57.

(23) Culture Medium Used to Maintain Nuclear Transfer Embryos

Also, contrary to the Office Action, a particular culture medium is not critical to the efficacy of the invention. Indeed, there are many different media that can be used interchangeably for growing bovine embryos in culture. The only important factor with

respect to such culture media is that they be based on a Krebbs-Ringer bicarbonate solution. This solution has a variety of salts and a bicarbonate buffer system that is based on the analysis of blood serum components. Such media generally contain an energy source, antibiotics and usually some complex component such as serum or co-cultured cells. In use currently for culture of bovine embryos are simple media, complex media, co-culture systems with cumulus cells, BRL cells or fibroblast cells, and completely defined media. (Different method in current usage can also be found in the Table in paragraph (22) supra.) Moreover, in my laboratory, one student is using a complex media with a cumulus cell co-culture while another student is using a simple media with a fibroblast co-culture. Both students are doing nuclear transplantation work and having success with development of embryos. The reason for using the different culture systems in the same lab simply is that one student is more comfortable with one system, and the other is more comfortable with the other system. Therefore, it is apparent that a specific culture media is not essential to the efficacy of the subject invention.

(24) Use of Method for Produce Other Cloned Species (Non-Bovines)

In my expert opinion, the subject cloning methods can be used to clone different mammals, i.e., other than bovines. That is to say, I am of the opinion that the basic discovery made by us that cells committed to a somatic lineage, preferably non-

quiescent cells, can be used as nuclear transfer donors to produce nuclear transfer embryos that give rise to viable fetuses and offspring, can be extrapolated to different, i.e., non-bovine mammals. In fact, subsequent to the filing of this patent application, I am aware that differentiated cells have reportedly been successfully used to produce cloned sheep and mice. (Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS, "Viable offspring derived from fetal and adult mammalian cells", *Nature,* 1997, 1385:810-813; Wakayama T, Perry ACF, Zucconi M, Johnsoal KR, Yanagimachi R., "Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei", *Nature,* 1998, 394:369-374)

(25) The generic nature of our discovery is further supported by recently reported experiments conducted by our lab relating to the efficacy of cross-species nuclear transplantation. Specifically, we conducted an experiment wherein we successfully produced a nuclear transfer fusion embryo by the insertion of an adult differentiated cell (obtained from the cheek of an adult human donor) into an enucleated bovine oocyte. This further substantiates the fact that adult differentiated cells of different species can be successfully "reprogrammed", notwithstanding the previous dogma that existed prior to the present invention, i.e., the widely-held but mistaken belief that only very early non-differentiated cells could be used for nuclear transfer donor cells or nuclei.

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(26) In summation, I hope that I have adequately addressed all of the Examiner's enablement concerns. For the reasons set forth above, and those enumerated in the present application, I am of the opinion that the efficacy of the subject invention does not depend upon the specific parameters, e.g., cell type, age, *in vitro* maturation, oocyte activation method, utilized in our working examples. Moreover, I believe that the Patent Office's conclusion is further untenable given the truly pioneering nature of the invention and further based on the fact that subsequent to the invention, differentiated cells have reportedly been successfully used by numerous groups to produce nuclear transfer embryos and cloned offspring.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 12/9/98 James M. Robl

James M. Robl, Ph.D.